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Award Number: W81XWH-08-1-0612

TITLE: Genetic Screen for PTSD-Prone Soldiers

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CONTRACTING ORGANIZATION:
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August, GA 30912-2300

REPORT DATE:
March 2010

TYPE OF REPORT:
Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: (Check one)

X Approved for public release; distribution unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
01/MAR/2010	Final	1 Sep 2008-28 Feb 2010

4. TITLE AND SUBTITLE

Genetic Screen for PTSD-Prone Soldiers

5a. CONTRACT NUMBER
W81XWH-08-1-0612

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Medical College of Georgia
Research Institute, Inc.
Augusta, GA 30912-2300

8. PERFORMING ORGANIZATION REPORT

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
US Army Medical Research
And Materiel Command
Fort Detrick
Maryland
21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release; distribution unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Genetic factors influence risk of exposure to trauma suggesting a role for personality traits like neuroticism, impulsivity, and/or preexisting conditions such as anxiety or depression in PTSD susceptibility. Calcyon is an excellent candidate gene for investigating a potential relationship between impulsivity and PTSD. We established a repository of cell lines from over PTSD-positive and control military personnel and veterans with mild to severe combat exposure. Each participant was extensively evaluated for trauma exposure, social support, medical history, personality traits and symptoms by clinicians specializing in PTSD, and genotyped for ancestry. We are on target for genotyping the calcyon gene in these subjects with respect to the C1 haplotype as it shows significant association with attention deficit hyperactivity disorder (ADHD). Single locus haplotype analyses of CAPS sores will be based on linear regression models including combat exposure, ethnicity, impulsivity and social environment as covariates. In addition, gene x environment interactions will directly be tested. All of our findings on the effect of polymorphisms in calcyon to PTSD susceptibility in the combat exposed military personnel will be submitted for publication, and if positive could be useful in prognostic screen of combat-worthy soldiers.

15. SUBJECT TERMS
calcyon; haplotype, association study; combat exposure; ethnicity; environment; covariates

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THISPAGE**			**19b. TELEPHONE NUMBER** *(include area code)*
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Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

Table of Contents

Page

INTRODUCTION

Post-traumatic stress disorder (PTSD) develops in a subset of patients exposed to traumatic stress. Twin studies with Vietnam Veterans revealed a role for genetic predisposition in trauma associated anxiety, re-experiencing trauma and avoidance of stimuli related to trauma (True et al., 1993;Goldberg et al., 1990). Genetic factors also influence risk of exposure to trauma. In particular, personality traits like neuroticism and impulsivity, and/or preexisting conditions such as anxiety or depression appear to play a role in developing PTSD (Lyons et al., 1993; Stein et al., 2002; Jang et al., 2003). A combination of data from human and murine studies supports the use of the calcyon as a strong candidate gene for investigating a potential relationship between impulsivity and PTSD (Trantham-Davidson et al., 2008; Laurin et al., 2005; Heijtz et al., 2007; Loos et al., 2009; Dasbanerjee et al., 2008) (CB, unpublished data). In particular, a DNA haplotype designated 'C1' in the calcyon locus appears to be strongly associated with both impulsive/hyperactive and inattentive features of attention deficit hyperactivity disorder (ADHD) (Laurin et al., 2005).

The goal of the current study is to determine whether the C1 haplotype could be useful as an unbiased screen for PTSD-prone soldiers. The association studies carried out thus far with other candidate genes do not support a strong basis for a genetic main effect in PTSD, and do not fully account for the genetic heritability of PTSD found in twin studies. Increasing evidence indicates that 'gene x environment' interactions (such as e.g., data on social support childhood abuse, or earlier diagnoses) should be included in genetic association studies. Our experimental design takes this into account in that our study involves an extensively phenotyped study population (see below). Further, if the data from the study funded by this award validate this hypothesis, haplotype analysis of the calcyon locus could constitute rapid, reliable genetic test for screening PTSD-prone soldiers prior to combat exposure. The availability of unambiguous and unbiased prognostic information on susceptibility to PTSD could prove invaluable in the assignment of soldiers to combat duty, possibly saving our country millions of dollars, and sparing the afflicted soldiers and their families, untold anguish.

BODY

Sub Aim 1. Obtain genomic DNA from cell lines established from 100 PTSD and 400 case control individuals.

The current study includes 328 subjects who are active military personnel and veterans with mild to severe combat exposure. Each participant is extensively phenotyped with respect to trauma exposure, social support, medical history, personality traits and symptoms by clinicians specializing in PTSD. The need for extensive evaluation of each subject to assess mental health history as well as social support slows the rate of recruitment due to the limited numbers of staff qualified to conduct and interpret results of such evaluations. On the other hand, the availability of this information is necessary in order to interrogate the role of co-variates such as environmental factors, personality type, and premorbid disorders in PTSD susceptibility or resilience. All subjects consented to genetic testing and provided a blood sample for DNA analysis. Cell lines for these individuals or controls had not been established at the outset of funding. Genomic DNA was isolated from lymphocytes from each subject and successfully transformed into lymphoblastoid cell lines to provide an unlimited source of DNA for the present study and future ones. All subjects also provided a urine sample for a toxicological screen.

Indeed, a key feature of our study design is that the subjects have been extensively interviewed and

evaluated by Dr. Baker, M.D., a specialist in PTSD. This process yielded valuable demographic information on the subjects as well data on ethnic background, medical history and symptoms. Specifically, each subject completed the following questionnaires: Fagerstorm test for nicotine dependence, DAST, AUDIT, SF-36 Health survey, Combat exposure Scale, Traumatic events survey-R, International Physical Activity, Beck Depression Inventory (BDI-II), Anger Scale - Retrospective Overt Aggression Scale, Cook-Medley Scale, STAXI-2, PDEQ-M, PDI-M, PPCQ-S, PSDQ, PLC-C, Cloninger personality Scale, and the CTQ. In addition, CAPS (clinician-administered PTSD scale), SCID, and HamD tests were conducted on all participants. All data was entered into a web-based databank and extensive data entry QC has been performed.

Analysis of the CAPS resulted in the following diagnoses: 31% with no PTSD (CAPS =30); 20% with non discordant phenotype (ndp) (CAPS 31-64); 49% with PTSD (CAPS >=65). PTSD scores significantly correlated with combat exposure (Spearman's rho =0.4, N=233, $p<0.001$). However, there was no correlation with gender, and the gender distribution was the same between the diagnostic groups (Table1).

Diagnosis	**Male (%)**	**Female (%)**
No PTSD	96	4
ndp	92	8
PTSD	91	9
Total	**93**	**7**

Table 1: Gender Distribution

Self-identified ethnicity and race was available only from 57% and 70%, respectively. Interestingly, these data showed that the ancestral background of the subjects was not evenly distributed between the diagnostic groups (Tables 2 and 3).

Diagnosis	**Hispanic**	**Non-Hispanic**	**NA**	**Total**
No PTSD	26	22	38	86
ndp	13	14	28	55
PTSD	49	32	54	135
Total	**88**	**68**	**120**	**276**

Table 2: Ethnicity of the Subjects in the Baker PTSD Study (based on 276 subjects)

Diagnosis	European American	African American	Asian	Native American	Hawaiian/ Pacific Islander	Other	NA	Total
No PTSD	47	2	6		2	3	26	86
ndp	32	2	3	2			16	55
PTSD	60	14	9	2	2	7	41	135
Total	**139**	**18**	**18**	**4**	**4**	**10**	**83**	**276**

Table 3: Race of Subjects in the Baker PTSD Study (based on 276 subjects)

Sub Aim 2. Genotype nine bi-allelic SNPs in the calcyon locus.

It is well known that population stratification, if not accounted for, will lead to false positive (and negative) findings in genetic association studies. Therefore, to strengthen our study, prior to genotyping of calcyon, we have genetically determined the ancestry of the study subjects using a small panel of ancestry-informative markers (AIMs). The publicly available genotype data including over 0.5M markers (Illumina 650y array) of 944 subjects of the Human Genome Diversity Panel (HGDP) (Li et al., 2008) was used as a reference population to select a set of 41 highly informative markers that were able to distinguish the 7 major world regions/continents (Nievergelt et. al, in prep.). These markers were selected to be suitable for multiplexing on the ABI SNPlex genotyping system (https://products.appliedbiosystems.com). Since the last report, ancestry determination was completed for most of the subjects in the present study. The figure below shows the actual ancestry determination in over 300 subjects for the Baker PTSD study.

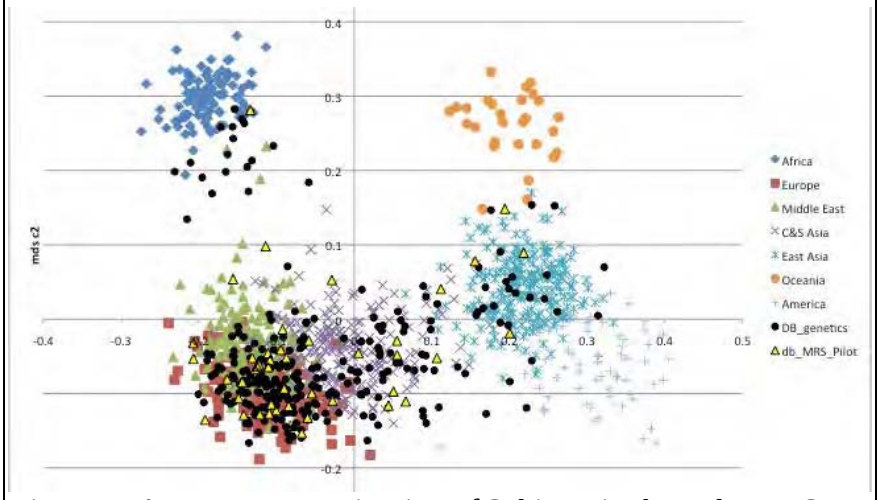

Figure 1: Ancestry Determination of Subjects in the Baker PTSD study (328 subjects included). Methods:41 markers (SNPlex), removed 11 for low genotyping, N=317 (genotyping rate: 0.997461). Included 944 HGDP subjects as Ancestral Populations of seven world regions indicated.

We also recently designed an ABI SNPlex genotyping panel with 45 SNPs covering five PTSD candidate genes, including four SNPs in calcyon. We plan to conduct 2 multiplexes of ~ 40 SNPs each.

The first SNP panel includes the following calcyon SNPs : 'rs2275723', 'rs4838721', 'rs41283333' and 'rs75840650.' Although the C1 haplotype is based on 9 bi-allelic SNPs, many of the SNPs in the haplotype are in high linkage disequilibrium (LD). Two of the SNPs we proposed to query on the chip , 'rs2275723' and 'rs4838721' SNPs of the C1 and C2 haplotypes, show significant transmission disequilibrium (TDT) with ADHD. Specifically, there is strong evidence that the C1 haplotype is overtransmitted in ADHD patients exhibiting either hyperactivity/impulsivity or inattention. In contrast, the C2 and C6 haplotypes show a tendency to an inverse relationship with the same symptoms. We also included 'rs75840650' as it is a functional SNP. In case there are problems or very promising results, we can repeat the genotyping with these SNP's and/or include additional ones on the second chip.

Sub Aim 3. Analysis and interpretation of genotyping data.
The reagents for these chips have been purchased and we anticipate completing the experimental aspect of the study within two months. The data will then be analyzed by Dr. Bergson, Rana and Baker as outlined in the original 'Statement of Work.' We expect it will take another 3-4 months to complete the data analysis and submit a manuscript summarizing the findings.

KEY RESEARCH ACCOMPLISHMENTS

- PTSD and control subjects included in the study were extensively evaluated to assess history of psychological/emotional conditions and social support. This information will permit assessment of important co-variates such as environmental factors, personality type, and premorbid disorders. This will facilitate testing 'gene X environment' interaction hypotheses regarding PTSD susceptibility related to combat exposure.

- Established cell lines from each participant for an unlimited source of subject DNA for future studies.
- Designed and validated an ancestry informative marker panel for 7 world regions/continents comprised of 41 SNPs.
- Determined ancestry of over 300 subjects. This information will permit assessment of ethnicity as a co-variate.

REPORTABLE OUTCOME

- Repository of cell lines from ~300 extensively phenotyped PTSD-positive and control combat exposed subjects.
- Developed and validated a 41 marker ancestry informative SNP panel.

CONCLUSION

We are on target to determine the C1 haplotype of the calcyon gene in over 300 subjects within the next four months. Single locus and haplotype analyses of CAPS sores will be based on linear regression models and will include combat exposure, ethnicity, impulsivity and social environment as co-variates. In addition, gene x environment interactions will directly be tested. All of our findings on the effect of polymorphisms in calcyon to PTSD susceptibility in combat exposed military personnel will be submitted for publication. If positive, this data could be useful as a prognostic screen of combat-worthy

soldiers.

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